Short-term response of the Ca cycle of a montane forest in Ecuador to low experimental $CaCl_2$ additions

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Abstract

The tropical montane forests of the E Andean cordillera in Ecuador receive episodic Sahara-dust inputs particularly increasing Ca deposition. We added $CaCl_2$ to isolate the effect of Ca deposition by Sahara dust to tropical montane forest from the simultaneously occurring pH effect. We examined components of the Ca cycle at four control plots and four plots with added Ca (2×5 kg ha^{-1} Ca annually as $CaCl_2$) in a random arrangement. Between August 2007 and December 2009 (four applications of Ca), we determined Ca concentrations and fluxes in litter leachate, mineral soil solution (0.15 and 0.30 m depths), throughfall, and fine litterfall and Al concentrations and speciation in soil solutions. After 1 y of Ca addition, we assessed fine-root biomass, leaf area, and tree growth. Only < 3% of the applied Ca leached below the acid organic layer (pH 3.5–4.8). The added $CaCl_2$ did not change electrical conductivity in the root zone after 2 y. In the second year of fertilization, Ca retention in the canopy of the Ca treatment tended to decrease relative to the control. After 2 y, 21% of the applied Ca was recycled to soil with throughfall and litterfall. One year after the first Ca addition, fine-root biomass had decreased significantly. Decreasing fine-root biomass might be attributed to a direct or an indirect beneficial effect of Ca on the soil decomposer community. Because of almost complete association of Al with dissolved organic matter and high free Ca^{2+} : Al^{3+} activity ratios in solution of all plots, Al toxicity was unlikely. We conclude that the added Ca was retained in the system and had beneficial effects on some plants.

Key words: Al speciation / calcium cycle / stem diameter growth / *Graffenrieda emarginata* Triana / tropical montane forest / molar Ca : Al ratio

1 Introduction

Although net primary production of tropical forests is thought to be mainly limited by N and/or P (*Tanner* et al., 1998; *Homeier* et al., 2012), Ca supply also can limit forest growth (*McLaughlin* and *Wimmer*, 1999; *Paoli* and *Curran*, 2007; *Boy* and *Wilcke*, 2008; *Baribault* et al., 2012). Calcium plays an essential role in regulating several physiological processes in plants like cell division, synthesis and function of membranes and cell walls, stomatal regulation, activation of the enzyme system involved in the plant response to environmental stimuli like low temperature, carbohydrate metabolism, disease resistance, and wound repair (*McLaughlin* and *Wimmer*, 1999).

A significant source of Ca for transatlantic ecosystems is Saharan mineral dust (*Kaufman* et al., 2005; *Boy* and *Wilcke*, 2008; *Pett-Ridge* et al., 2009b). *Pett-Ridge* et al. (2009a) reported that 83% of the atmospheric Ca input in a watershed in Puerto Rico originated from Saharan dust and only 17% from sea salt. In S Ecuador, *Boy* and *Wilcke* (2008) demonstrated that during a strong La Niña event in 1999/2000, Ca and Mg which originated in the Sahara, were deposited from the atmosphere to the east-exposed slope of the E Andean cordillera. *Boy* and *Wilcke* (2008) hypothesized that the Ca supply by Saharan-dust deposition is linked with the El Niño Southern Oscillation (ENSO) which might change as an effect of global climate change (*Timmermann* et al., 1999; *Richardson* et al., 2009). A consequence could be altered Ca supply to the Ca-poor north Andean tropical montane forest growing on Palaeozoic bedrock.

The response of tropical montane forest to nutrient inputs can either be determined by assessing changes in the nutrient cycling or by observing plant growth and change of plant morphological properties. Retention of nutrients deposited from the atmosphere in the canopy was interpreted as indicating a scarcity of the considered nutrient (*Ulrich*, 1983). In a

montane forest of S Ecuador, *Boy* and *Wilcke* (2008) observed Ca retention in the forest canopy during elevated Ca deposition from the atmosphere while Ca was leached during low Ca deposition. They suggested that Ca was a colimiting nutrient and that the biological retention of Ca was triggered by one or several unknown other nutrients that are also deposited with elevated Ca inputs.

In the response of transatlantic forest ecosystems to Sahara dust–derived Ca depositions, the potential Ca effect, however, cannot be separated from the simultaneously occurring pH effect because Ca is at least partly deposited as carbonate salt as indicated by elevated pH values of rainfall (*Boy* and *Wilcke*, 2008). The resulting near-neutral pH in the soil solution likely stimulates organic-matter (OM) turnover and thus the release of organically bound nutrients, such as N. *Soethe* et al. (2006) observed a nonsignificant increase in fine-root growth in an Ecuadorian tropical montane forest after the addition of lime to an ingrowth core. Again, it was not possible to separate the effects of Ca and pH. To study the Ca effect alone, a pH-neutral salt such as $CaCl_2$ was added experimentally in a temperate forest by *Hugget* et al. (2007), who found long-term effects of Ca on growth and health of sugar maple. *Baribault* et al. (2012) observed a correlation of the growth of tropical tree species with high wood density with base-cation concentrations in soil, while growth of trees with low-wood-density trees was correlated with P concentrations.

In acid mineral soils, Ca deficiency and Al toxicity can appear simultaneously (*Marschner*, 1995; *Schaedle* et al., 1989), which makes it difficult to distinguish between both growth-limiting factors. The total Al concentration in soil solution often failed to describe Al toxicity most probably because of the differential toxicity of the various Al species (*Kinraide*, 1991). While the free Al^{3+} ion and polynuclear Al species (*e.g.*, $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ which is in the literature referred to as Al_{13}) are phytotoxic, the toxicity of mononuclear $Al(OH)^{2+}$ and $Al(OH)_2^+$ ions is under debate. There are indications for the toxicity of fluoride complexes (AlF^{2+} and AlF_2^+), while sulfate complexes and organically complexed Al are not toxic (*Alva* et al., 1986; *Savory* and *Wills*, 1991; *Cronan* and *Grigal*, 1995, *Kinraide*, 1997). The strongly toxic polynuclear Al species (*e.g.*, Al_{13}, *Kinraide*, 1991) can form in solution if fast changes of pH or Al concentrations occur and Al concentrations are high enough ($> 10^{-5}$ mol L^{-1}; *Furrer* et al., 1992). However, in the thick organic layers of tropical montane forest soils, where most roots are located (*Soethe* et al., 2006), dissolved Al concentrations are comparatively low and likely almost entirely organically complexed because of high dissolved organic carbon (DOC) concentrations.

We determined the short-term response of the Ca cycling over four Ca additions and the response of plant performance after two to four Ca additions to an old-growth lower montane forest in S Ecuador by adding Ca as a neutral salt at a low rate to simulate Sahara-dust deposition. Because of the small Ca addition we (1) hypothesized that there were little changes of the Ca cycle during the observation period. Furthermore, we hypothesized that (2) added Ca remains in the ecosystem as indicated by insignificant Ca leaching to greater soil depths but that nevertheless added Ca (3) reduces plant nutrient stress because Ca is at least colimiting and (4) alleviates Al toxicity.

2 Materials and methods

2.1 Study area

The studied forest is located in S Ecuador on the E slope of the Cordillera Real of the Andes (*i.e.,* the E cordillera) at an altitude between 2020 and 2120 m asl in the Reserva Biológica San Francisco in the deeply incised valley of the Rio San Francisco draining to the Amazon (3°59′ S, 79°05′ W). The vegetation of the study site can be classified as "evergreen lower montane forest" (*Homeier* et al., 2008). In the study area, > 300 tree species have been identified so far with Lauraceae, Melastomataceae, and Rubiaceae as the most abundant plant families (*Homeier* and *Werner*, 2007). *Graffenrieda emarginata* Triana is the most common tree species on our study plots contributing about one quarter of all stems (28.3%) having a dbh ≥ 10 cm. The mean stem diameter, the mean number of trees, and stem basal area per plot (prefertilization survey of trees ≥ 10 cm dbh) were 14.6 cm, 45.3, and 0.85 m^2, respectively. Average stand height was 12 to 14 m. The estimated crown radius for canopy trees was between 2 and 4 m, some of the biggest trees may have reached 5–6 m. There is no data available about the root radius of canopy trees.

The mean annual rainfall between 1999 and 2009 measured at 2–4 gauging stations with five rain collectors at each station was (2400 ± 180 [SD]) mm. Annual bulk Ca depositions with rainfall ranged between 0.90 and 13 (mean: 4.0) kg ha^{-1} y^{-1} for the period 1999–2009. In 2008, it was 2.5 kg ha^{-1} y^{-1} and in 2009, it was 5.1 kg ha^{-1} y^{-1}, respectively. Mean annual temperature at 1950 m asl is 15.2°C (*Bendix* et al., 2008). The soil is a Stagnic Cambisol (Hyperdystric, Chromic) (*IUSS Working Group WRB*, 2007) developed from Palaeozoic phyllites, quartzites, and metasandstones.

2.2 Experimental design

The study site was located on the upper slope near a major ridge and has an average slope of 51% (range: 25% to 84%). The experiment consists of Ca addition and unfertilized control plots. Each treatment was fourfold replicated. The unfertilized control plots were located upslope to avoid nutrient leaching from fertilized to control plots. Three replicates of the Ca treatment and control had a N aspect and the fourth a S-SW aspect. The location of each plot was selected in a way that the vegetation was representative of the area and similar on all plots. Each plot was 400 m^2 (20 m × 20 m) large, and the distance between the plots was at least 10 m.

The Ca-addition plots received 10 kg Ca ha^{-1} y^{-1} (along with 17.7 kg Cl^- ha^{-1} y^{-1} which is approximately the same as the mean total annual deposition in the two study years of 15.6 Cl^- ha^{-1} y^{-1}) split in two applications per year. The Ca addition is equivalent to approx. 2.5 times the mean annual Ca deposition with rainfall of 4.0 kg ha^{-1} y^{-1} between 1999 and 2009. Additions of Ca started in January 2008, followed by three further applications in August 2008, February 2009,

and August 2009. Calcium was applied manually in solid phase as a fine-grained $CaCl_2 \cdot 2H_2O$ salt in *pro analysi* quality by four persons walking parallel in strips of 5 m. Throughfall collectors and collecting bottles of litter percolate were covered with plastic bags during Ca application. The research equipment (except throughfall collectors) was installed in subplots of 2 m × 2 m. Six subplots were located along two perpendicular random-transects in the 20 m × 20 m plots and the transects were at least 4 m from the plot border so that the subplots were located at least 2 m from the plot border to avoid edge effects. Although the application of $CaCl_2$ increased Cl^- fluxes in all soil solutions significantly, it did not change the electrical conductivity of the soil solution in the root zone (approximated by litter leachate, averaged over the 2 years of $CaCl_2$ application: (64 ± s.d. 16) $\mu S\,cm^{-1}$ in the control and 75 ± 8 in the Ca treatments). Therefore, we assumed no effect of Cl^- on plant performance. Even the highest Cl^- concentration of 18.6 mg L^{-1} measured on a single date in one of the Ca treatments is far below known plant physiological effects of Cl^- because toxicity to Cl-tolerant plants only occurs above Cl^- concentrations of 700 mg L^{-1} (*Marschner*, 1995).

2.3 Soil sampling

Before the first nutrient application in August 2007, we sampled the Oa, A, and B horizons of each plot from hand-dug profiles (where we later installed the zero-tension lysimeters) within 2 weeks. Bulk density of the soil was measured by the soil core method (*Blake* and *Hartge*, 1986) for each horizon at the four soil profiles. After two nutrient applications (October 2008), the Oi, Oe, Oa, A, and B horizons were resampled with an Edelman auger. On both sampling dates, we collected soil to a depth of about 0.5 m in the mineral soil. Furthermore, the horizons of the O layer were sampled in a representative way per plot after the third fertilizer application between April and June 2009. The soil samples were transferred to plastic bags and transported to the laboratory, where processing of the stored samples (4°C) took place within 6 weeks.

2.4 Plant sampling

To assess fine-root biomass, we took six root samples per plot from the O layer with a soil corer (3.5 cm ∅) in January 2009. The soil samples for fine-root analysis were soaked in water and cleaned from soil residues using a sieve with a mesh size of 0.25 mm. Only fine roots (root diameter < 2 mm) of trees were considered for analysis. Live fine roots (biomass) were separated from dead rootlets (necromass) under the stereomicroscope based on color, root elasticity, and the degree of cohesion of cortex, periderm, and stele. A dark cortex and stele, or a white, but nonturgid cortex, or the complete loss of the stele and cortex with only the periderm being present, were used as indicators of root death (*Persson*, 1978; *Leuschner* et al., 2001). The fine root biomass of each sample was dried at 70°C for 48 h and weighed. The data were expressed as fine-root abundance (g m^{-2}).

Fine litterfall was collected once per month from six litter traps (0.60 m × 0.60 m) per plot from October 2007 until March 2008. Leaf samples from sun-exposed branches of the four most common tree species (*A. lojaense, G. emarginata, H. fendleri,* and *Myrcia* sp. nov.) from 4–5 individual trees per treatment and species were collected in January 2009 to quantify changes in leaf morphology and foliar nutrient concentrations one year after the first fertilization. We sampled 10–20 fully developed leaves without visible damages from two different branches of each tree.

2.5 Ecosystem solution sampling

Rainfall was collected with five funnel gauges at two measurement stations on forest clearings in the study area. Throughfall was collected with 20 fixed-positioned funnel gauges in each plot (*Wullaert* et al., 2009). Three zero-tension lysimeters per plot were used to collect leachate from the O layer. They were made of plastic boxes with a 0.15 m × 0.15 m collection area and covered with a polyethylene net with a mesh size of 0.5 mm. Soil solution at the 0.15 and 0.30 m mineral-soil depths was collected with suction lysimeters which had a ceramic cup with 1 μm pore size. Based on our visual inspection, we estimate that the total fine-root fraction in the O layer is > 95% so that the collected soil solution can be considered as from below the rooting zone. Throughfall, litter leachate, and mineral soil solution were sampled fortnightly. After each sampling, we applied a vacuum of –0.6 bar to the suction lysimeters in order to collect sufficient sample for the next sampling period. Sampling of throughfall, litter percolate, and soil solution took place from August 2007 until December 2009 of which the first five months were before the first nutrient addition. The observation period covered four Ca applications.

2.6 Field measurement of plant properties

We monitored stem diameters of 362 trees with dbh > 10 cm in the study plots with permanent girth-increment tapes (D1 dendrometer, UMS, Munich, Germany) at breast height (1.3 m) which were read in 6-week intervals. We calculated relative diameter increments (*i.e.,* diameter increments normalized to the initial tree diameter) for the four most common tree species to take into account the influence of stem diameter on its increment from February 15, 2008 (after the first Ca addition) until January 24, 2010 (after four Ca applications).

Per leaf sample, 10–25 fresh leaves were scanned using a flatbed scanner (CanonScan LIDE 30, Canon). Subsequently, images were analyzed with the software WinFolia 2001a (Regent Instruments Inc., Canada) for calculation of the average single leaf area (LA). Leaves were then dried at 60°C to constant mass, and specific leaf area (SLA) was calculated as the ratio of LA to leaf dry weight. The leaf-area index (LAI) was quantified with two LAI-2000 plant canopy analyzers (LI-COR Inc., Lincoln, NE, USA). The LAI measurements were conducted in the remote mode, *i.e.,* by synchronous readings below the canopy at 2 m height above the forest floor and in a nearby open area ("above-canopy" reading) using two devices. One measurement was done above each litter trap and a second at the same time outside the for-

est. The LAI measurements were realized in January 2008 (before the first fertilization) and in January 2009 (1 year after the first fertilization). All measurements were conducted during periods of overcast sky. To avoid reductions in the sky sector seen by the LAI-2000 fish-eye lens (by high mountains or trees at the horizon), only data of the three inner rings (0°–43° from zenith) were analyzed.

2.7 Chemical analyses

Soil and fine-litterfall samples were dried at 40°C to constant mass after field collection, the mineral soil was sieved to < 2 mm. Soil and litterfall samples were ground with a ball mill. Ground leaf and litterfall samples were digested with 65% HNO_3 in p.a. quality under pressure in a microwave oven (MARS Xpress, CEM GmbH, Kamp-Lintfort, Germany).

Soil pH was measured in a soil-to-deionized water mixture (ratio of 1:10 and 1:2.5 for the organic horizons and mineral soil, respectively). To measure the effective cation-exchange capacity (ECEC) of mineral soil samples, 5 g of sample were shaken in 100 mL unbuffered 1 mol L^{-1} NH_4NO_3 for 1 h, then filtered and analyzed for Al, Ca, K, Mg, and Na concentrations. Base saturation (BS) was calculated as the percentage of the sum of charge equivalents of the base metal cations of the effective CEC.

After collection in the field, throughfall, litter leachate, and soil-solution samples were transported to our field laboratory where first electric conductivity (ProfiLine Cond 3110, WTW GmbH, Weilheim, Germany) and then pH (Sentix HWS, WTW GmbH, Weilheim, Germany) were immediately measured in an aliquot of each sample within < 24 h. Another aliquot was filtered (ashless filters with pore size 4–7 μm, folded filter type 389; Munktell & Filtrak GmbH, Bärenstein, Germany) and frozen until transport to Germany for further analysis. During transport to Germany, samples were stored cool at < 4°C and transport did not take longer than 1 week.

Concentrations of Ca in litterfall digests, Ca and Al in water samples and Al, Ca, K, Mg, and Na in soil extracts were determined with flame atomic-absorption spectrometry (AAS, AA240FS, Varian, Darmstadt, Germany or ZEEnit 700P, Jena Analytik, Jena, Germany), while Ca and Al concentrations in leaf digests were determined with inductively coupled plasma–optical emission spectroscopy (ICP-OES, Optima 5300 DV, Perkin Elmer, Rodgau, Germany). Water samples were furthermore analyzed for concentrations of total dissolved Cl^-, NH_4^+, NO_3^-, and PO_4^{3-} using continuous-flow analysis (CFA, Bran+Luebbe GmbH, Norderstedt, Germany). Sulfate was determined by ion chromatography (IC, Dionex ICS-900, Thermo Scientific, Waltham, MA, USA). Fluoride concentrations were determined with an ion-sensitive electrode (WTW Inolab pH/Ion 735 with a WTW F800 electrode, WTW, Weilheim, Germany) after addition of TISAB III (Fluka Analytical) by standard addition, because of the lower detection limit compared with IC. Total organic C concentrations (TOC) in litter leachate were measured with a high-temperature TOC analyzer (high TOC *II*, Elementar Analysensysteme, Hanau, Germany).

To assess the precision of the Ca, K, Mg, Na, and TOC analyses, we used an internal reference standard (a throughfall water sample from our field site in Ecuador) in every laboratory run of ≈ 100 samples. The relative standard deviations of Ca, K, Mg, Na, and TOC concentrations in the reference standard were 11%, 2.7%, 5.5%, 27%, and 8.6%, respectively (n = 15 for the base metals and 25 for TOC). The relative standard deviation of Na was elevated because the Na concentration in the reference solution was close to the detection limit of 0.009 mg L^{-1}.

2.8 Aluminum speciation

The speciation of Al in litter leachate was modeled with measured data for solution composition (Cl, PO_4^{3-}, NH_4^+, NO_3^-, K, Na, Mg, Ca, DOC, pH, Al, SO_4^{2-}, and F^- concentrations and ionic strength) using the geochemical code Visual MINTEQ (VMINTEQ, Version 3.0 beta, J.P. Gustafsson). Within VMINTEQ, the NICA-Donnan model was used to assess complexation of Al with humic substances. Details about the NICA-Donnan model are given in *Kinniburgh* et al. (1996). NICA-Donnan parameters of metal complexation of organic acids were taken from the literature (*Milne* et al., 2003), an active DOM : DOC ratio of 1.65 was assumed, and the dissolved organic acids were assumed to be 80% fulvic and 20% humic acids (*Tipping* et al., 2002). Strongly toxic polynuclear Al species (*e.g.,* Al_{13}, *Kinraide*, 1991) are not considered in the model. However, Al_{13} was not supposed to exist in solution in the forest soil because no strong changes in Al concentrations or pH occurred and total dissolved Al concentrations were generally low (mean 16.4, range 1.4–94 μmol L^{-1}).

The Al speciation was modeled for 228 samples, for which complete data sets of all relevant ion concentrations were available. The F^- concentrations were determined for a selected number (n = 176) of litter leachate and mineral soil solutions and were in all cases below the limit of quantification of 0.022 mg L^{-1} and in 89% of the cases even below the limit of detection of 0.007). For the calculation we set F^- to the detection limit (0.007 mg L^{-1}), to avoid underestimation of fluoro-Al complexes. Sulfate concentrations were available only for a limited number of samples (n = 31). Mean SO_4^{2-} concentration was 0.61 mg L^{-1} (range 0.19–1.4 mg L^{-1}). For all samples without measured SO_4^{2-} concentrations, values in the model were set to 0.61 mg L^{-1} (*i.e.,* the mean sulfate concentration). Ionic strength was estimated from electrical conductivity according to *Griffin* and *Jurinak* (1973).

2.9 Calculations

To calculate reference evapotranspiration with REF-ET (University of Idaho and R.G. Allen; version 2.0) using the ASCE Penman-Monteith method based on daily mean wind speed, air humidity and irradiation, daily precipitation sum and daily minimum and maximum temperature, we used meteorological data obtained from the automatic meteorological station on a clear-cut area in ridge-top position at 1950 m asl. Instrumentation is described in *Wullaert* et al. (2010). Data were obtained from Rollenbeck et al. (pers. comm.). Leaching losses from the O layer were calculated using a one-dimen-

sional soil-water-balance model based on *DVWK* (1996) and *Oelmann* et al. (2007) as described for our study sites in Ecuador by *Wullaert* et al. (2010).

To set up the canopy budget of Ca, we used the model of *Ulrich* (1983) in the way described by *Wullaert* et al. (2010) based on Cl^- as tracer. In earlier work at the same study site, it was confirmed that Cl^- can indeed be considered as inert in the aboveground part of the study ecosystem (*Boy* and *Wilcke*, 2008; *Boy* et al., 2008; *Wilcke* et al., 2009). To reduce the impact of spatial and temporal heterogeneity of dry deposition, time lags between deposition on plant surfaces and washoff with throughfall and the known high uncertainty of Cl^- concentration measurements in electrolyte-poor solutions, we used mean deposition ratios of the whole study period to estimate dry deposition of Ca. We did not detect temporal trends in fortnightly Cl^--deposition ratios suggesting that the canopy properties and dry-deposition rates did not change significantly during the observation period. Deposition ratios of Cl^- ranged from 1.06 to 1.85 in the control and 1.25 to 2.12 in the Ca treatments.

2.10 Statistical analyses

To test for differences in fine-root biomass between the Ca and control treatments, the six subplots were considered as independent replicates because the distance between the sample locations was large enough. We used a one-factorial nonparametric Kruskal-Wallis test followed by a post-hoc paired comparison (Wilcoxon-U-test).

To test if there were *a priori* differences in Ca concentrations and fluxes in throughfall, litterfall, litter leachate, and soil solution between the Ca and control treatments, the time period before the first nutrient application was assessed. The whole period after the first nutrient application was analyzed separately from the period before the first nutrient application. Changes in Ca concentrations in throughfall, litterfall, litter leachate, and soil solution were analyzed by linear mixed effects models. We used this type of model since it allowed us to analyze the data set even when several data were missing because of, *e.g.*, a lack of sufficient amount of sample to analyze. The treatment was defined as fixed effects and the four plot replicates as random effects.

For soil parameters, LAI, tree diameter growth, leaf morphology, and foliar Ca and Al concentrations, the effects of Ca application were assessed using a *t*-test for independent data sets. Significance was set at $p \leq 0.05$. All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA), except statistics of root-biomass data which was performed with SAS software (version 8.2, SAS Institute, Cary, NC, USA).

3 Results

3.1 Soil solid phase

The mean Ca concentrations in the Oi horizon increased in the Ca treatment after three Ca applications significantly ($p = 0.013$). Consequently, the mean molar ratio of total Ca/Al concentrations in the Ca treatment increased significantly after three Ca applications in the Oi horizon ($p = 0.037$). Storages of Ca in the organic layer ranged between 40 and 359 kg ha^{-1}. Thus, the annual Ca addition represented 2.8%–25% of the Ca stored in the O layer. Our application rate of 10 kg ha^{-1} y^{-1} had a similar size as the storage of (7.3 ± 1.9 [SD]) kg ha^{-1} Ca in the upper 0.3 m of the mineral soil in the Ca treatment. The mean storages of total Ca in the O layer and exchangeable Ca in the mineral soil were not significantly different between control and Ca treatments.

3.2 Calcium fluxes

There were no significant differences in mean annual water fluxes between the Ca and control treatments during the whole observation period (Fig. 1). All ecosystem fluxes of Ca were higher in the second than in the first year (Fig. 2). This concurs with a higher bulk Ca deposition with rainfall of 5.1 kg ha^{-1} in 2009 than in 2008 with only 2.5 kg ha^{-1}. Before nutrient application, the Ca concentrations and fluxes in in all eco-

Table 1: Selected soil properties of the Ca and control treatments (means ± SD; $n = 4$) in the study area determined in October 2008 (mineral soil) after two Ca applications and in April–June 2009 (O layer) after three nutrient applications.

Property	Control treatment	Ca treatment
O layer		
Oi horizon		
pH range (1:10 H_2O)	4.64–5.08	4.67–5.24
Ca / mg g^{-1}	1.9 ± 0.3	3.6 ± 0.9 *
Molar Ca : Al ratio	0.58 ± 0.09	1.5 ± 0.5 *
Oe horizon		
pH range (1:10 H_2O)	3.86–4.63	3.90–4.97
Ca / mg g^{-1}	0.81 ± 0.44	2.2 ± 1.3
Molar Ca : Al ratio	0.13 ± 0.08	0.42 ± 0.35
Oa horizon		
pH range (1:10 H_2O)	3.50–3.90	3.39–3.75
Ca / mg g^{-1}	0.16 ± 0.17	0.16 ± 0.13
Molar Ca : Al ratio	0.033 ± 0.027	0.033 ± 0.031
Mineral soil		
A horizon		
pH range (1:2.5 H_2O)	3.67–3.81	3.69–3.80
ECEC / $mmol_c$ kg^{-1} [a]	26 ± 10	41 ± 13
BS / % [b]	3.6 ± 1.5	2.3 ± 1.1
B horizon		
pH range (1:2.5 H_2O)	4.01–4.20	4.04–4.14
ECEC / $mmol_c$ kg^{-1} [c]	37 ± 2	37 ± 5
BS / % [d]	0.9 ± 0.3	0.9 ± 0.1

[a] Effective cation-exchange capacity; [b] base saturation; * data significantly different from control treatment ($p < 0.05$).

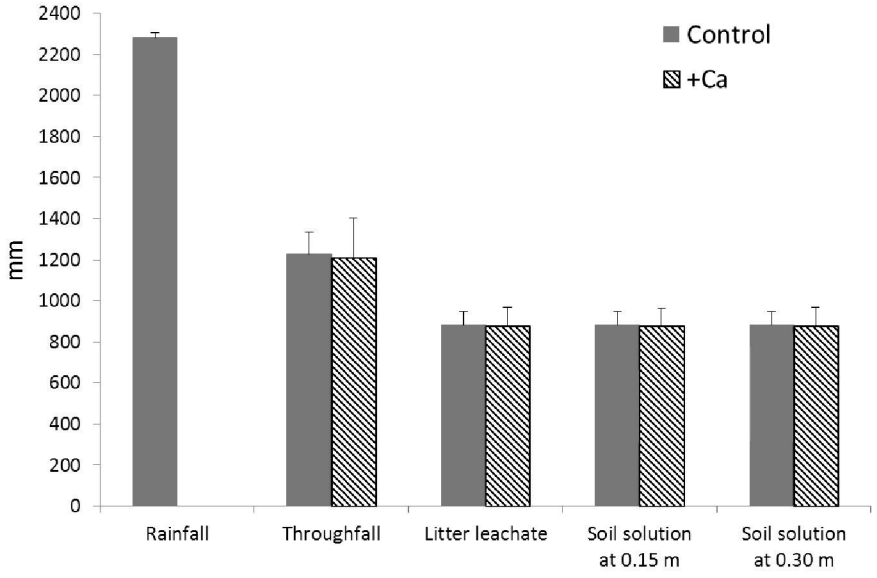

Figure 1: Mean elements of the annual water budget of control and Ca treatments during two hydrological years (2008 and 2009). Error bars represent standard deviations of five replicate rainfall collectors and the four plots of control and Ca treatments for all other fluxes.

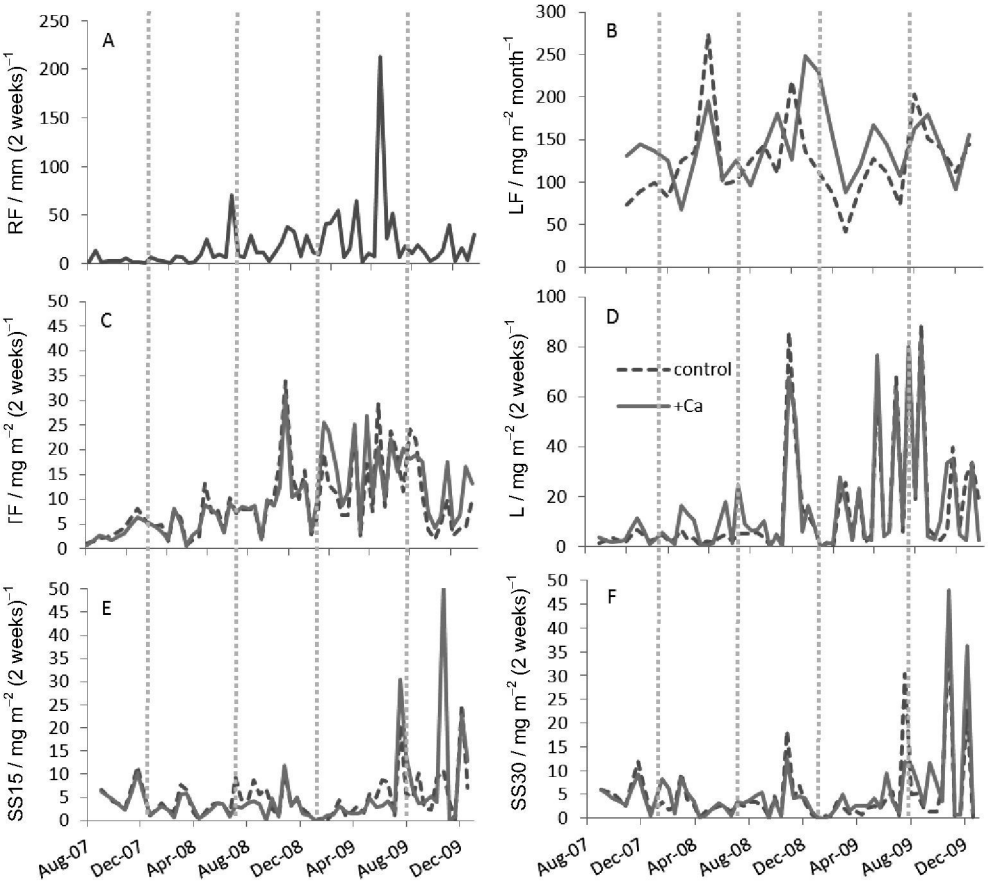

Figure 2: Mean (A) rainfall and mean Ca fluxes in (B) litterfall (LF), (C) throughfall (TF), (D) litter leachate (LL), (E) mineral soil solution at 0.15 m depth (SS15), and (F) mineral soil solution at 0.30 m depth (SS30) in control and Ca treatments between August 2007 and December 2009. Calcium application dates are indicated by vertical gray dotted lines.

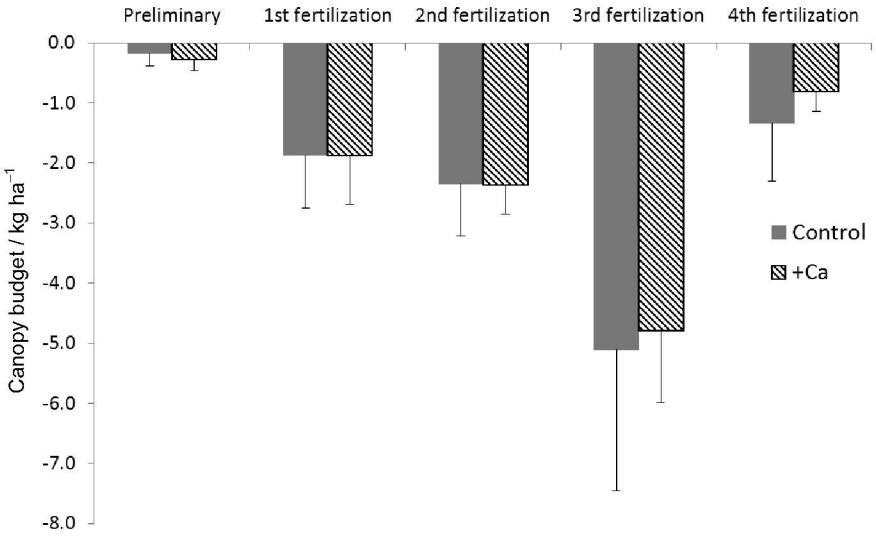

Figure 3: Mean canopy budget of Ca for the preliminary phase and the four fertilizer periods (see text for exact dates). Negative values of canopy budget indicate net retention in the canopy. Error bars represent standard deviations of the four replicate plots per treatment. The canopy budget was calculated according to *Ulrich* (1983) from Ca fluxes in bulk deposition and throughfall using Cl^- as an inert tracer.

system solutions did not differ significantly between the Ca and control treatments (not shown).

Our Ca addition did not change any ecosystem flux of Ca significantly during the observation period of 2 y (Fig. 2), although our Ca-application rate of 10 kg ha^{-1} y^{-1} was high compared with the 1.5–1.7 kg ha^{-1} y^{-1} cycled with litterfall, 2.6–2.9 kg ha^{-1} y^{-1} leached from the O layer, 1.2–1.3 kg ha^{-1} y^{-1} leached to 0.15 m soil depth, and 1.1–14 kg ha^{-1} y^{-1} leached to 0.3 m soil depth.

Dry deposition contributed (2.1 ± 1.5) kg ha^{-1} Ca in 2008 and 2.9 ± 2.1 in 2009 in the control and 2.3 ± 1.9 and 3.3 ± 2.6, respectively, in the Ca treatments. In all plots, Ca was retained by the canopy from total deposition (Fig. 3). The retention accounted for (62 ± 25)% of the totally deposited Ca during the whole study period in the control treatments and (59 ± 8.1)% in the Ca treatments. While in the preliminary phase and the first year of the experiment, Ca retention was not different between control and Ca treatments, in the second year Ca retention tended to be reduced (albeit not significantly) in the Ca treatment compared with the control plots.

3.3 Plants

After two Ca applications, mean living fine-root biomass (370 g m^{-2}) was significantly lower (16.5%; $p < 0.05$) in the Ca than the control treatment (Fig. 4). At the same time, mean dead fine-root biomass tended to be lower in the Ca treatment but was not significantly different from control (400 *vs.* 425 g m^{-2}). Accordingly, the mean live-to-dead ratio in fine root mass was lower in the Ca than the control treatment.

After four Ca additions, the mean cumulative tree-diameter increment tended to increase slightly in the pooled rare species and in two of the four most common tree species (*G. emarginata, H. fendleri*) but did not differ significantly between Ca and control treatment. In contrast, the mean cumulative tree-diameter increment of A. *lojaensis* and *Myrcia* sp. nov. was smaller in the Ca than the control treatment. The reduced growth of *Myrcia* sp. nov. was significant ($p = 0.014$, Tab. 2). Total fine-litterfall production in the Ca treatment ([4.4 ± 0.9 (SD)] t ha^{-1} y^{-1} in 2008 and 4.3 ± 0.6 in 2009) was not significantly different from that of the control treatment (4.3 ± 0.8 in 2008 and 4.5 ± 0.8 in 2009).

The mean LAI increased between January 2008 and January 2009 from 4.72 ± 0.77 to 4.80 ± 0.92 in the Ca treatment and from 4.60 ± 0.93 up to 4.69 ± 1.01 in the control treatment, but the mean LAI increment was not significantly different between the Ca and control treatments. The average LA of the three of the four most common tree species (*G. emarginata, H. fendleri,* and *Myrcia* sp. nov.) was smaller 1 y after the first Ca application in the Ca than the control treatment but changes were only significant in *Myrcia* sp. nov. (Tab. 3). Mean concentrations of Ca and Al in fresh leaves did not differ significantly between the Ca and control treatments (Tab. 3).

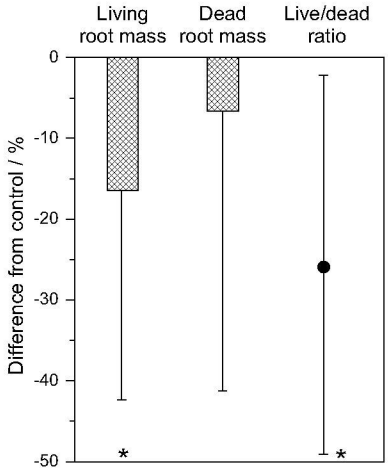

Figure 4: Living and dead fine-root biomass. Data in the Ca treatment is presented as percent deviation from the control treatment. A star (*) represents that the mean in the Ca treatment is significantly different from the control treatment. Data kindly provided by Dietrich Hertel. Error bars represent standard deviation.

Table 2: Means and ranges of diameter at breast height (dbh) on February 15, 2008 and cumulative tree-diameter increments from February 15, 2008 to January 24, 2010 of the four most common tree species and the pooled rest of the tree species relative to the diameter on February 15, 2008. Errors are standard deviations.

	***Alchornea lojaensis* Secco**	***Graffenrieda emarginata* (Ruiz & Pav.) Triana**	***Hieronyma fendleri* Briq.**	***Myrcia* sp.nov.**	**Pooled "rare" species**
Control					
number of trees	22	48	8	17	73
Mean dbh /cm and range on February 15, 2008	15.3 ± 6.5 (9.9–37.0)	12.4 ± 2.3 (9.6–18.5)	13.1 ± 2.7 (10.2–19.0)	12.6 ± 3.0 (9.6–21.7)	15.8 ± 5.3 (9.4–34.1)
Cumulative tree diameter increment (/ %)	0.79 ± 1.16	1.66 ± 1.38	0.23 ± 0.19	1.59 ± 0.99	0.41 ± 0.75
Ca treatment					
number of trees	19	54	18	19	84
Mean dbh /cm and range on February 15, 2008	18.0 ± 9.8 (10.6–55.4)	14.0 ± 3.1 (10.0–25.9)	12.7 ± 2.6 (9.3–19.4)	13.4 ± 3.3 (9.7–19.9)	15.5 ± 4.6 (9.7–26.3)
Cumulative tree diameter increment / %	0.45 ± 0.73	1.70 ±1.47	0.46 ± 0.95	**0.81 ± 0.80***	0.59 ± 0.72

* Mean significantly different from control treatment ($p < 0.05$).

Table 3: Mean foliar Ca and Al concentrations, Ca : Al ratios, leaf area (LA), and specific leaf area (SLA) in fresh leaves of the four most common tree species in early February 2010, two years after the first Ca addition. Errors are standard deviations.

	***Alchornea lojaensis* Secco**	***Graffenrieda emarginata* (Ruiz & Pav.) Triana**	***Hieronyma fendleri* Briq.**	***Myrcia* sp.nov.**
Control				
number of trees	5	5	5	5
Ca / mg g^{-1}	3.25 ± 1.05	1.42 ± 0.20	1.68 ± 0.56	0.82 ± 0.30
Al / mg g^{-1}	0.20 ± 0.12	3.24 ± 0.89	0.00 ± 0.00	0.06 ± 0.01
Ca : Al ratio [a]	16 ± 10	0.31 ± 0.07	71	10 ± 3
LA /cm^2	31.8 ± 11.5	178.3 ± 44.7	21.2 ± 7.1	20.2 ± 4.2
SLA /cm^2 g^{-1}	37.9 ± 4.6	42.2 ± 2.2	66.7 ± 10.9	39.6 ± 2.9
Ca treatment				
number of trees	4	5	4	6
Ca / mg g^{-1}	4.62 ± 0.97	1.45 ± 0.41	1.87 ± 0.44	1.00 ± 0.27
Al / mg g^{-1}	0.22 ± 0.04	3.60 ± 1.02	0.01 ± 0.02	0.04 ± 0.03
Ca : Al ratio [a]	15 ± 5	0.29 ± 0.10	374 ± 350	28 ± 25
LA /cm^2	30.1 ± 5.7	201.4 ± 68.5	24.3 ± 4.9	15.3 ± 3.1*
SLA /cm^2 g^{-1}	43.2 ± 2.9	43.4 ± 3.8	62.2 ± 8.6	37.9 ± 3.0

[a] Molar ratio as suggested by *Cronan* and *Grigal* (1995); * mean significantly different from control treatment ($p < 0.05$).

3. 4 Speciation of aluminum

To assess the possible Al toxicity, we modeled the chemical speciation of Al in litter leachate, which reflects the root environment. Our chemical speciation modeling revealed that in the O layer the mean toxic free Al^{3+} and other possibly toxic inorganic Al complexes accounted only for < 2% of the total Al concentrations, while > 98% of the Al was bound to dissolved organic matter (Tab. 4). At low DOC concentrations, potentially toxic Al species can account for up to 42% of the total Al concentration. However, the number of samples with dissolved inorganic Al > 10% of the total Al was small (3.8% of all samples) and in 88% of the samples dissolved inorganic Al concentrations were below 1% of total Al. The activity ratio of Ca^{2+} : Al^{3+} was only in one sample < 1, while all other samples showed ratios > 1 reaching up to 1987.

4 Discussion

4.1 Calcium cycling

Although our annual Ca application was high relative to all considered ecosystem fluxes (generally < 3 kg ha^{-1} y^{-1}), it

Table 4: Aluminum speciation and Ca : Al ratios in the litter leachate of control plots and the Ca treatment.

	Al total	Al^{3+}		Other anorganic Al[a]		Organically bound Al[b]		Ca : Al	Ca^{2+} : Al^{3+}	Ca^{2+} : Al^{3+}
	/ μmol L^{-1}	/ μmol L^{-1}	/ %	/ μmol L^{-1}	/ %	/ μmol L^{-1}	/ %	molar ratio	concentration ratio	activity ratio
Overall (n = 228)[c]										
Mean	16.4	0.18	0.81	0.05	0.26	16.2	98.94	0.72	56.9	61.4
Min.	1.39	0.00	0.00	0.00	0.01	0.81	57.74	0.02	0.69	0.75
Max.	94.1	15.33	37.14	2.90	6.67	76.1	99.99	8.60	1846	1987
Control (n = 102)[c]										
Mean	15.5	0.26	0.82	0.06	0.26	15.2	98.0	0.71	67.3	72.6
Min.	4.14	0.00	0.00	0.00	0.01	4.1	71.4	0.05	1.50	1.63
Max.	94.1	15.33	24.66	2.90	3.91	76.1	100.0	4.39	1846	1987
Ca treatment (n = 108)[c]										
Mean	16.7	0.13	0.97	0.04	1.20	16.60	97.8	0.83	53.1	57.2
Min.	1.39	0.00	0.00	0.00	0.01	0.81	57.7	0.02	0.69	0.75
Max	32.3	5.74	37.14	0.80	6.67	33.3	100.0	8.62	285	304

[a] Refers to all potentially toxic inorganic Al species other than Al^{3+}; [b] refers to all species bound to organic matter (> 90% of Al^{3+} was electrostatically bound to the Donnan phase); [c] differences in total number of samples and sum of samples from control and Ca treatments are attributable to the fact that the overall number of samples also included a few samples taken before the first Ca addition.

did not change any ecosystem flux significantly (Fig. 2). Possibly, the effect of Ca addition on the Ca fluxes was hidden by the high variation in Ca storages in the O layer among the replicate plots of both treatments (coefficients of variation among replicates of the control plots in April–June 2009: 13% and of the Ca treatment: 79%). The Ca losses to the deeper subsoil were small (Fig. 2) which is also reflected by the fact that the base saturation of the A horizon did not differ between the soil samples collected prior to the first Ca application and those collected after two Ca applications (Tab. 1), which should have been the case if a significant portion of the added Ca had been leached given the similar size of the annual application rate and storage of exchangeable Ca in the top 0.3 m of the mineral soil. Because of the low pH of the O layer which only contains variable charge, it is not likely that the Ca was mainly retained by adsorption to negatively charged surfaces, instead biological uptake into microorganisms and plants must have been the major retention mechanism.

The lack of significant changes in the considered ecosystem fluxes might be related with the short duration of the experiment of 2 y. The cumulative Ca application only amounted to 5%–50% of the Ca storage in the O layers in the first 2 y of the experiment which were completely accumulated in the system. For the future, we expect that increasingly less Ca will be retained which likely causes significant changes in the Ca fluxes.

Calcium was retained in the canopy during the whole observation period in control and Ca treatments suggesting a Ca demand of the canopy trees, their epiphytes and/or epiphylls (Fig. 3), as previously reported by *Boy* and *Wilcke* (2008). Because we did not study the Ca budget of the different components of the canopy (*i.e.*, canopy plants, epiphytes, epiphylls) we cannot rule out that the demand of Ca by one or more of the components overcompensates the leaching of one or more of the other components.

The addition of Ca to the soil tended to decrease the uptake of Ca by the canopy from total deposition (*i.e.*, wet and dry deposition) from the third Ca application on although the differences in the canopy budgets of Ca between Ca and control treatment were not significant until the end of our study period. This indicates that our Ca application allowed the vegetation to cover an increasingly larger part of its Ca requirement by uptake from the soil. A similar observation was made for the application of N and P which both also reduced canopy retention of these nutrients (*Wullaert* et al., 2010). However, it remains to be seen whether this apparent effect continues to occur in future and finally becomes significant.

Based on the Ca fluxes with litterfall and the additional Ca cycling with throughfall, at least 21% of the added Ca was taken up by the plants and recycled to the soil. To this percentage the unknown amount of Ca accreted in wood must be added. In red spruce, Ca can occupy around 60%–80% of the cation-exchange sites in the xylem (*Momoshima* and *Bondietti*, 1990). A nonfertilized Puerto Rican rain forest accreted 2.4–5.1 kg y^{-1} Ca (*McDowell* and *Asbury*, 1994) which would correspond to approx. 25%–50% of added Ca in our study.

4.2 Plant performance

Nutrient limitation results in morphological and physiological adaptations such as, *e.g.*, root-to-shoot ratio, slow growth rate, increased leaf longevity, dense and shallow root mats,

and small trees with thickened leaves. Living fine-root biomass in the organic layer was reduced in the Ca treatment compared with the control (Fig. 4). This suggests that the trees invested less C (and energy) in fine-root mass because the additional Ca improved the growth conditions. Alternatively, root distribution might have changed in a way that more roots entered the mineral soil. Decreased fine-root necromass is attributable to the relatively high fine-root turnover (*Graefe* et al., 2008a, b). While we can rule out a detrimental effect of the Cl^- addition associated with the fertilized Ca because Cl^- concentrations remained far below known effect levels and also did not change the electrical conductivity of the litter leachate, there are several possible explanations for the plant responses to Ca addition: (1) Tree growth was limited by Ca deficiency (*Baribault* et al., 2012). (2) The microbial community was limited by Ca deficiency and our Ca addition stimulated its activity resulting in enhanced nutrient release, particularly of N, to soil solution which improved nutrient supply of the plants. (3) The plants suffered from Al toxicity which was alleviated by the Ca addition. We hypothesize that on the long run, we will observe significantly increased tree growth which was the case in a forest in Canada 3 y after Ca addition as $CaCl_2$ (*Huggett* et al., 2007).

The significant negative response of the relative cumulative tree-diameter increment of *Myrcia* sp. nov. to the Ca application illustrates that not all trees benefited from increased Ca availability (Tab. 2). Similarly, *A. lojaenses* also tended to a negative response while the other two species tended to a positive response. This illustrates that there will be winners and losers among the tree species in response to increased Ca availability just like was observed in response to increased N and/or P availability by *Homeier* et al. (2012).

4.3 Calcium limitation of trees or microbial communities in the forest floor

There is only limited information about the effect of Ca on productivity of forest ecosystems. *Hugget* et al. (2007) reported long-term effects on multiple parameters related to tree growth and health in a Ca-fertilization experiment with sugar maple. *Baribault* et al. (2012) detected a correlation of base-cation concentrations in soil with the growth rates of tropical tree species with high wood density, while growth of trees with low wood density was correlated with soil P concentrations. These results suggest that Ca directly promoted the growth of at least some tree species. *Kaspari* et al. (2008) investigated the response of the decomposer community of a tropical lowland forest in Panama to the addition of a cocktail of nutrients including Ca and found enhanced leaf-litter decomposition. Although it was not possible to determine which of the applied elements caused the observed response, the results illustrate the possibility that the addition of Ca enhances leaf-litter decomposition and that the stimulated release of other nutrients than Ca explains the response of at least some of the trees.

4.4 Alleviation of Al toxicity

In acid soils, Al becomes a strong competitor of Ca and interferes with Ca uptake in roots and root growth (*McLaughlin* and *Wimmer*, 1999). Many of the symptoms of Ca deficiency are associated with Al toxicity (*Edwards* et al., 1976). One factor limiting forest growth might therefore be Al toxicity. *Cronan* and *Grigal* (1995) reported in their review several threshold conditions to detect Al stress in forests including (1) soil BS < 15% of effective CEC, (2) molar Ca : Al concentration ratio < 1 in soil solution, and (3) a molar foliar tissue Ca : Al concentration ratio ≤ 12.5.

After two Ca applications at our study plots, the BS in the A horizon of the Ca treatment was still very low with (2.3 ± 1.1)%, *i.e.,* clearly < 15% (Tab. 1). The fact that the majority of the tree roots were located in the thick O layer might therefore be an adaptation in order to avoid Al toxicity in the mineral soil. Our speciation modeling of Al in litter leachates indicated that toxic Al species were hardly present (mean 0.23 μmol L^{-1}; 1.1% of total Al) in the soil solution of the O layer. Maximum Al^{3+} concentrations reached up to 15.3 μmol L^{-1} in a few samples with low DOC concentrations, while on average > 97% of the Al was organically bound and thus supposed to be nontoxic (*Kinraide*, 1997). *Wheeler* et al. (1992) reviewed the Al tolerance of 34 plant species (including 87 cultivars) growing in diluted nutrient solutions and concluded that in order to reduce the shoot dry weight by 50%, for the most sensitive genotypes Al^{3+} concentrations > 1 μmol L^{-1} and for more tolerant genotypes > 30 μmol L^{-1} were necessary. Our modeled mean free Al^{3+} activities were usually far below 1 μmol L^{-1}. However, in some samples maximum Al^{3+} concentrations were > 1 μmol L^{-1} and might therefore have induced toxic effects for most sensitive species. However, only 3% of all studied samples had Al^{3+} concentrations > 1 μmol L^{-1}. Thus, the Al concentrations in 97% of our litter-leachate samples should not be toxic even for the most sensitive plants, and for more tolerant genotypes, toxic Al^{3+} concentrations were never reached (Tab. 4). The mean molar Ca : Al ratio of the litter leachate was 0.72 (range 0.02–8.61) and thus in a range for which Al toxicity could be assumed (*Cronan* and *Grigal*, 1995). However, the free ion activities of Ca^{2+} : Al^{3+} were on average 56.9 (range 0.69–1846). Thus, Al speciation in the litter leachate suggested that Al toxicity was unlikely.

Both, *A. lojaense* and *H. fendleri* had higher molar Ca : Al ratios than 12.5 before and after four additions of Ca. In *H. fendleri*, the Ca : Al ratio rose significantly after Ca addition while it remained almost unchanged in *A. lojaense* (Tab. 3). The high molar Ca : Al ratios in both *A. lojaense* and *H. fendleri* did not indicate Al toxicity. *Graffenrieda emarginata* is an Al accumulator with much higher Al concentrations than all other studied species. *Myrcia* sp. nov. had a foliar molar Ca : Al concentration ratio slightly below the toxicity threshold proposed by *Cronan* and *Grigal* (1995) before the Ca addition but clearly above after four Ca additions (Tab. 3). Because at the same time the diameter growth of *Myrcia* sp. nov. responded negatively to the Ca additions (Tab. 2) in spite of the apparent alleviation of Al toxicity by Ca addition, we suggest that Al toxicity is not an issue in the study area, which is consistent with our finding that only a minor fraction of the total dissolved Al in soil solution occurs as toxic Al species (Tab. 4).

5 Conclusions

The addition of 10 kg ha^{-1} y^{-1} did not significantly influence any nutrient flux after 2 y although it was markedly higher

than all individual Ca fluxes. We attribute this result to the high variation in Ca storage of the O layer which in the 2 y of the experiment prevented changed Ca fluxes to be recognized with our approach. The fact that nutrient leaching through soil was unaffected by the Ca addition demonstrates that the added Ca was held in the upper part of the ecosystem (aboveground biomass and organic layer). Reduced fine-root biomass after only 1 y of Ca application revealed a fast plant response indicating a generally improved nutrient supply of the plants in the Ca treatment compared to the control. However, there were also negative responses of plant performance illustrating that not all plant species will benefit from improved Ca availability. The low Al concentrations and the high free Ca^{2+} : Al^{3+} activity ratios in litter leachate and the molar Ca/Al concentrations in plant tissue (with the exception of the Al-accumulating *G. emarginata*) suggest that there is no Al toxicity. This is probably attributable to the almost complete organic association of dissolved Al in the soil solution of the O layer both before and after Ca addition in all plots. We therefore suggest that the positive response of the vegetation to Ca application is not attributable to an alleviation of Al toxicity. From these results, we conclude that deposited Ca will be retained in the forest and have differential effects on the tree species of the study ecosystem resulting in winners and losers among the species.

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